APPENDIX B
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FIG. 2 - Affinity purification of a recombinant protein based on peptide-peptide complexes isolated from the genetic assay. Scheme for purification of GST-13-mer (SEQ ID NO:5) by affinity chromatography using immobilized MBP-LEP27 (SEQ ID NO:6) (Zhang et al., 2000).

FIG. 5 - "Far Western" blot protocol to eliminate false positives. An extract is prepared from phage-resistant cells that express the DBD-LEP fusion. This is electrophoresed through a denaturing gel, then blotted onto a membrane. This is probed with a GST fusion protein containing the target epitope, followed by labeled anti-GST antibody to visualize the site(s) of binding of the GST-peptide fusion protein. In this case, two of the four selected peptides provided strong signals, showing that they are true hits, while two (LEPC and LEPD, provided weak or unobservable signals, indicating that they are false positives, or binding peptides that associate too weakly to be of practical utility. LEPA = Large (40 kD) polypeptide; LEPB = KARKEAELAAATAEQ (SEQ ID NO:2); LEPC = PCP; LEPD = PCHLNCSLQTLSPTRTTPRKHCKHCFKTLSEKMKWN (SEQ ID NO:3).

FIG. 9 - Experimental demonstration of substrate-targeted inhibition of proteolysis using an epitope-binding peptide (LEPB). The experiment shown schematically in FIG. 5 was carried out. Addition of increasing amounts of LEPB to a reaction containing a protease and two different substrates inhibited one cleavage event, but not the other. Only the substrate containing the epitope recognized by LEPB was protected from cleavage. GG = GSTNEAYVHDGPVRSLNGFP (SEQ ID NO:3); GM = GSTGVVWFKDSVGVSGNMBP (SEQ ID NO:4); LEPB = KARKEAELAAATAEQ (SEQ ID NO:1).

As a second step to ensure that repression is dependent on the presence of the DBD-target peptide expression plasmid, the inventor will cure the cells of the target-encoding plasmid and verify that efficient repression no longer occurs. Initially, the inventor did this by gel separation of the linearized library- and target-encoding plasmids, followed by religation of the library-

encoding plasmid and transformation into fresh cells, but this is tedious. The inventor has placed a unique FseI site (recognition sequence 5′-GGCCGGCC-3′) (SEQ ID NO:7) in the target plasmid, allowing it to selectively linearize the target plasmid in a mixed preparation that also contains the library-encoding plasmid. The DNA preparation will then be treated with T4 DNA polymerase in the absence of dNTPs to allow the voracious exonuclease activity of this enzyme to degrade the linearized target-encoding DNA. The resultant DNA will be retransformed into fresh cells and the transformants will be streaked onto X-gal plates (the receptor strain employed in this assay, JH372, has an integrated lacZ gene under the control of a lambda operator (Hu *et al.*, 1990)). The vast majority of cells will now contain only the library-encoding plasmid, so any survivors that make white patches at this point will be discarded. Of course, this protocol will result in the loss of a true positive if the library DNA contains a FseI site. But this will occur very infrequently since it is an eight base pair site and is comprised entirely of C's and G's.